Development of a One-Handed Environmental Surface Sampling Device

Daniel J. Angelini[1], Kyle Ford[1], Kristy L. Williams[2], Peter Emanuel[1], Calvin Chue[1]

[1]Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, [2]Excet, Inc., Springfield, VA

INTRODUCTION

Currently, there are several biological surface sampling kits that are designed to acquire and safely transport biological specimens. In these kits, many of the items are individually packaged and require both hands for optimal use. Since many of these items are packaged individually, the operator will generate a large amount of waste that will need to be managed during the course of a sampling mission. We have designed a sampling device (Figure 1) to facilitate biological sampling using one-hand and reduce waste materials, which we have named the Mano Sampling Device. The advantage of this device versus currently available sampling technology is that this device acts as the actual sampler as well as the transport packaging, therefore, speeding up sampling times and reducing waste. The one-handed operation of the device will simplify sampling while wearing mission oriented protective posture (MOPP) gear in a hazardous environment. This design indicates a major step forward in biological sampling technology.

Figure 1

Figure 1: Mano Sampling Device.

MATERIALS AND METHODS

Design for Manufacturing: The current design was evaluated for manufacturability, and an outer sanitary container/packaging was added to ensure that sampling surfaces are not compromised prior to use.

Test Kit Production: ECBC's Advanced Design and Manufacturing (ADM) team produced 25 sampling kits, which allowed for the collection of 125 samples.

Evaluation of the Collection Device: The Gram-positive organism, *Bacillus atrophaeus* var. *globigii* (Bg), (Unified Culture Collection [UCC] designation: BACI051) was selected as a surrogate for the well known biothreat agent *Bacillus anthracis* Ames. This bacterial stock was obtained from the Critical Reagents Program (Frederick, MD). We examined the effectiveness of the new sample collection device using two different test surfaces (12"x12" glass or stainless steel). The selected concentrations of spores (10^7 or 10^5) were spotted on test surfaces and allowed to dry for ≥1 hour. Samples were collected in 15mL phosphate buffer with 0.05% Tween 20 (w/vol) according to the modified CDC-defined method (http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html). The samples were then spread plated on TSA plates, incubated at 37°C overnight, and evaluated for colony growth. The results were expressed as mean ± standard deviation (SD) of the % colony forming units (CFU) recovered. Finally, similar experiments were performed using the Biological Sampling Kit (BiSKit; NSN 6550-01-541-6661) as a standard control. For the final report, we will repeat each condition (surface type, spore concentration) with an n ≥ 3 on 3 separate days to ensure statistical significance.

DNA Extraction: DNA extraction and purification will be performed using the UltraClean Microbial DNA Isolation Kit [MoBio Laboratories, Inc.; Carlsbad, CA] according to the manufacturer's recommended protocol on the remaining collection buffer following plating.

Quantitative Real Time (qRT)-PCR Evaluation of Purified Nucleic Acid: PCR was performed to ensure compatibility with currently used PCR reagents/methods. Extracted DNA samples were run for PCR amplification in an ABI 7900HT Sequence Detection System (Life Technologies; Carlsbad, CA). Experimental samples were run at a concentration of 0.02ng/µL; 5µL of DNA sample will be added to 15µL of master mix. The samples were then cycled in two stages. Stage 1 consists of 1 cycle at 50°C for 2min and 1 cycle at 95°C for 20sec. Stage 2 consists of 45 cycles at 95°C for 1sec, followed by 60°C for 20sec. The FAM/TAMRA-labeled primers for BACI051 will be obtained through the Critical Reagents Program and used as directed. RT-PCR results for BACI051 will be considered positive if the cycle threshold (Ct) value was <40; negative results will be considered to be Ct values of >40 or absent Ct values. Results will be reported as mean ± SD of resultant Ct values.

RESULTS

Figure 2

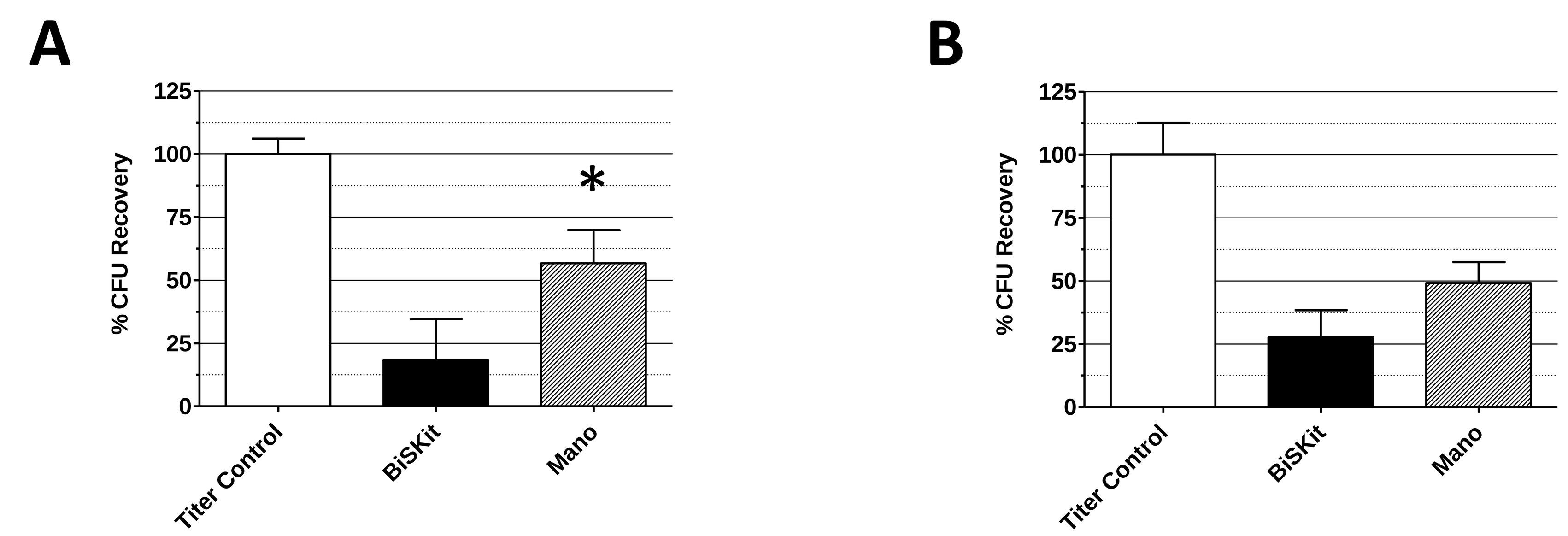

Figure 2: Collection efficiencies for the BiSKit and Mano Collection Devices on Glass Surfaces. Each 12" x 12" glass surface was inoculated with either (A) 10^7 or (B) 10^5 Bg spores. The spores were then recovered from the collection devices as stated in the Materials and Methods section. Results are displayed as the mean ± SD of the % of total CFU recovery.

Figure 3

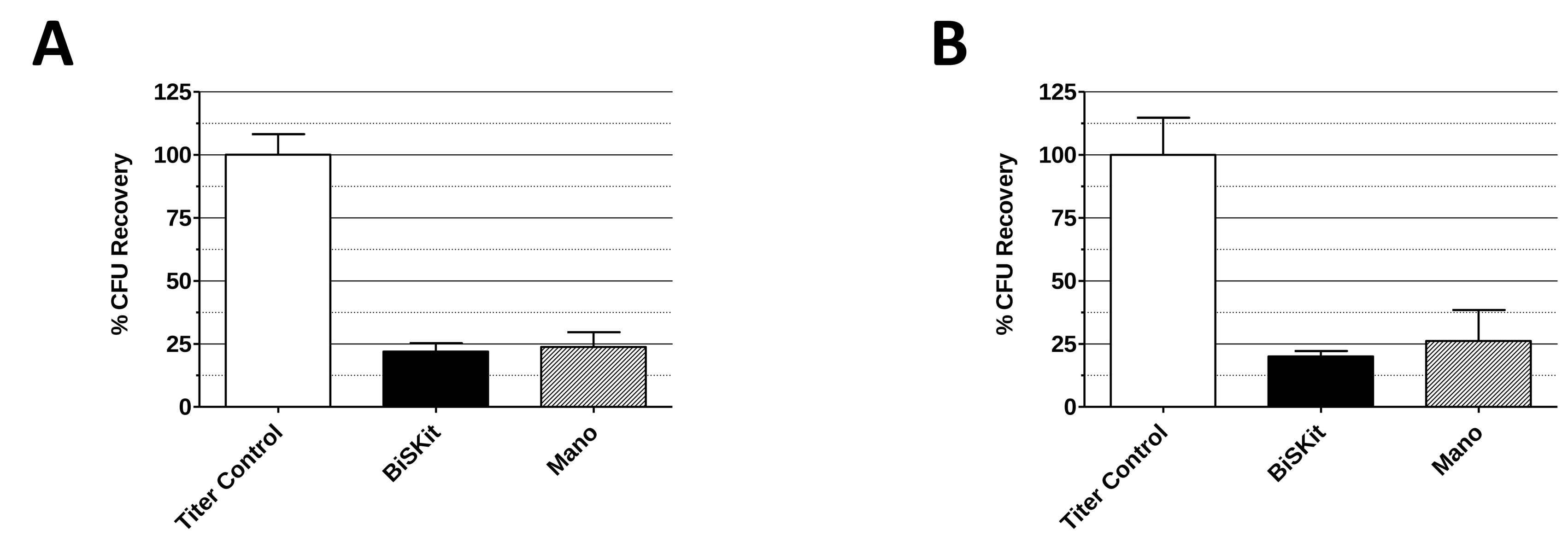

Figure 3: Collection efficiencies for the BiSKit and Mano Collection Devices on Steel Surfaces. Each 12" x 12" stainless steel surface was inoculated with either (A) 10^7 or (B) 10^5 Bg spores. The spores were then recovered from the collection devices as stated in the Materials and Methods section. Results are displayed as the mean ± SD of the % of total CFU recovery.

Figure 4

	Ct Values		
		Glass (10^7)	Steel (10^7)
Titer Control	27.31 ± 2.97		
BiSKit		29.00 ± 2.54	30.45 ± 2.87
Mano		26.06 ± 0.65	28.05 ± 0.93

Figure 4: PCR results following collection of 10^7 Bg spores off glass or stainless steel surfaces with either the BiSKit or Mano. Nucleic acid extraction and PCR were performed as stated in the Materials and Methods section. Results are displayed as the mean ± SD of the Ct values for each condition.

CONCLUSIONS

The initial results of this study indicate that the Mano Sampling Device is capable of collecting a *B. anthracis* simulant with greater efficiency than the currently employed BiSKit device in a laboratory setting. Also, this study demonstrates that the Mano is capable of collecting samples that are compatible with currently used PCR technologies. Overall, these results indicate that the Mano should be evaluated further. These studies should include a larger sample size, different surface materials, various microorganisms (e.g. Gram-negative bacteria, viruses), and toxins. These results could be used to create a new reference method for environmental surface sampling. This work was funded through the ECBC's 219 Program.